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RESEARCH EFFORTS

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Conjugated Catecholamines

The direct measurement of norepinephrine (NE) and epinephrine (E) in the bloodstream of man is currently the most useful technique for evaluating sympathetic nervous activity. Measurements of NE and E are particularly useful in controlled laboratory situations, where the speed with which they respond to sympathetic nervous activity can be used to advantage. However, this rapidity is a drawback when measuring sympathetic activity in subjects under real life stressful circumstances. NE and E are very labile molecules, which deteriorate if samples are not handled carefully. Their half life in the body is only about 2 minutes, so that blood samples must be obtained during a stressful procedure. Samples taken after that stress has already occurred will have decreased catecholamine levels and will seriously underestimate the degree of sympathetic nervous activation. It is thus not practical to use plasma catecholamines as a guide to the overall level of sympathetic nervous activation during space flight. Even if samples were taken very quickly after stress, the short half life of catecholamines swiftly decreases levels of circulating NE and E and the variable half life of these amines between subjects does not allow extrapolation back to what the actual peak might have been. It is possible to calculate the circulating half life of catecholamines in a given individual at low catecholamine levels, since clearance is primarily dependent on neuronal uptake, referred

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to as $uptake_1$. However, when there are extremely high levels of circulating catecholamines, uptake into the vascular cell wall ($uptake_2$), becomes a prominent phenomenon. Under these circumstances, the half life of the catecholamines may be much briefer at high levels than at low levels. Man can attain extremely high levels of circulating catecholamines in response to stress; a subject can increase his circulating NE level from 300 to 15,000 pg/ml simply by running to exhaustion.

In an effort to circumvent the extreme lability of plasma catecholamines and find a measure of sympathetic activity applicable to subjects in a wider range of activities, we have investigated indices of sympathetic nervous system activity which are not so transient. Dopamine-beta-hydroxylase (DBH) is the enzyme which converts dopamine into norepinephrine and is released with NE from sympathetic nerve endings. Blood levels of this enzyme increase after stress in experimental animals and man, and it has a half life estimated at several days in man. DBH appears to be a useful index of sympathetic nervous activity during repeated or severe stress, but it changes only minimally in response to minor stress.

These difficulties in evaluating sympathetic nervous activity in real life situations could be overcome with a less transient index of sympathetic activity than catecholamines, but not quite so stable and insensitive to small changes as DBH. The primary metabolites of catecholamines could provide such an index (Fig. 1).

FIGURE 1

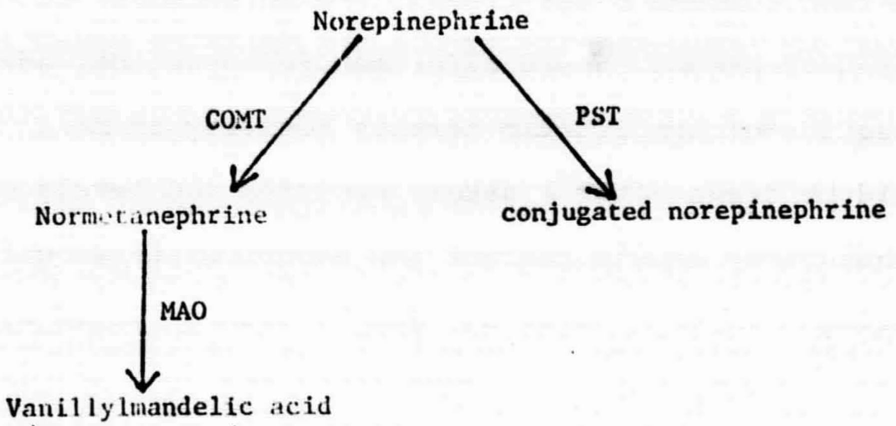


Fig. 1: Metabolism of norepinephrine.

COMT catechol-O-methyltransferase

PST phenylsulfotransferase

MAO monoamine oxidase

The enzyme phenylsulfotransferase (PST) sulfates catecholamines. The sulfated catecholamines are then actively excreted by the kidney and this active mode of excretion gives them a half life of about 2 hours. We modified the technique described by Buu and Kuchel in 1977 to allow measurement of these conjugated catecholamines in blood, urine and cerebrospinal fluid. The measurement of conjugated catecholamines in plasma should offer several practical advantages to the evaluation of sympathetic nervous activity in man. Blood samples could be drawn after a stress occurred and levels of conjugated catecholamines should reflect the amount of these catecholamines released during the recent stress. Sample storage would be greatly simplified as the conjugated catecholamines are more stable in blood than are catecholamines themselves. Since the half life of the conjugated catecholamines is longer than that of the parent catecholamine, it would not be necessary to take repetitive blood samples during a stress to evaluate the total amount of sympathetic nervous response to stress.

The modification of the technique of Buu and Kuchel to measure conjugated catecholamines in plasma relies on hydrolysis of these conjugates by acid and heat. Our technique for deconjugation of these substances in cerebrospinal fluid and urine relies upon lyophilization, as does the Buu and Kuchel technique, but at only 1/100th the concentration of acid that they report, thus avoiding the problem

of destroying the sample during deconjugation procedures. Specifics of the plasma deconjugation method are as follows:

Adjust plasma to 0.3 M $HClO_4$ and 0.1 mM dithiothreitol.

Centrifuge at 50,000 g x min.

Transfer 100 µl of the supernatant to a 10 x 130 mm sealing Sarstedt® plastic tube, seal with the cap and transfer to test tube rack.

Secure the tops with a flat plate and shield the bottom of the tubes with aluminum foil so that they cannot directly absorb heat and infrared radiation in an oven.

Incubate in an oven at 95°C x 45 min.

Take out of the oven and immerse the bottom 10 mm of the tube in ice water to condense any water vapor present.

Remove the caps and proceed to assay by the COMT technique of Durrett and Ziegler.

Deconjugation of Catecholamines in Cerebrospinal Fluid or Urine

Take 100 µl of cerebrospinal fluid or 100 µl of 1:10 dilution of urine and adjust to 0.003 M $HClO_4$.

Lyophilize at less than 10 microns x 16 hrs.

Reconstitute to 100 µl with water.

Assay by COMT technique of Durrett and Ziegler.

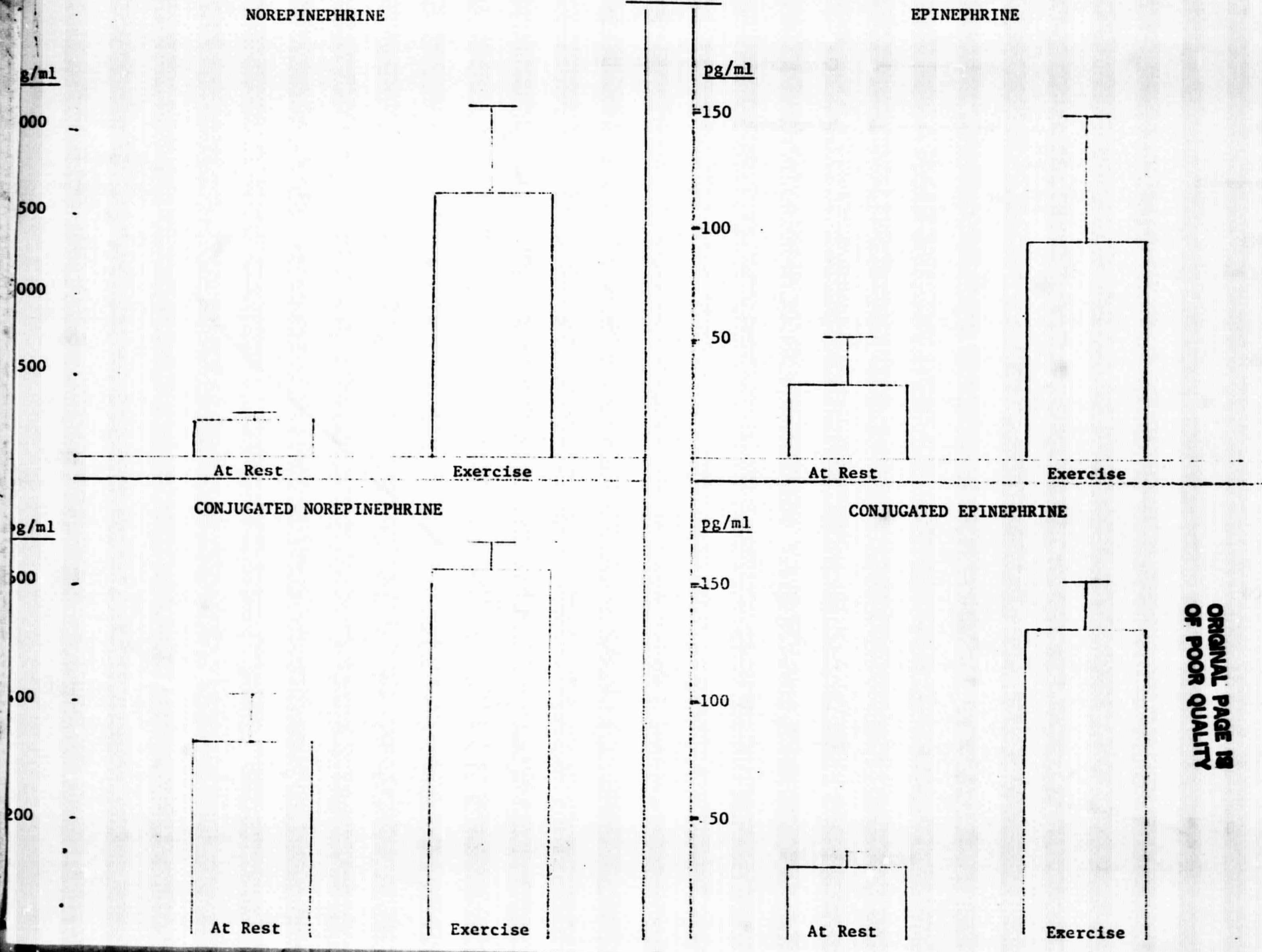
The amount of conjugated catecholamines present in a sample is equal to the total catecholamines in the deconjugated sample minus the free catecholamines present in the unprocessed sample. By this technique, conjugated dopamine levels are about 10 to 100 times as high as free dopamine levels, conjugated E levels are about 5 times as high as free E levels, and conjugated NE level is about 1.5 times free NE levels. The relative levels of free and conjugated NE and E in subjects at rest and following exercise are shown in Fig. 2.

The utility of this technique for evaluating sympathetic nervous response to stress after the stress has occurred is shown in Fig. 3. Figure 3 illustrates that exercise markedly increases plasma levels of NE during exercise, but 10 minutes after exertion, NE levels fall back nearly to normal range. In contrast, conjugated NE levels increase as circulating levels of NE are processed by phenylsulfotransferase and converted to conjugated NE.

Relationship of Plasma Norepinephrine to Levels of Severe Stress

We use plasma NE levels as a measure of stress in man, but their actual relationship to a given level of stress has not been very precisely mapped out. In Fig. 4, plasma NE levels increased markedly during the final stages of exhaustive exercise in a group of 8 men as their heart rate increased toward a theoretical maximum of 200 beats per minute. A more useful representation of this data is shown in Fig. 5, where NE levels are plotted on a logarithmic

FIGURE 2


NOREPINEPHRINE
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100
50
At Rest
Exercise

Figure 3

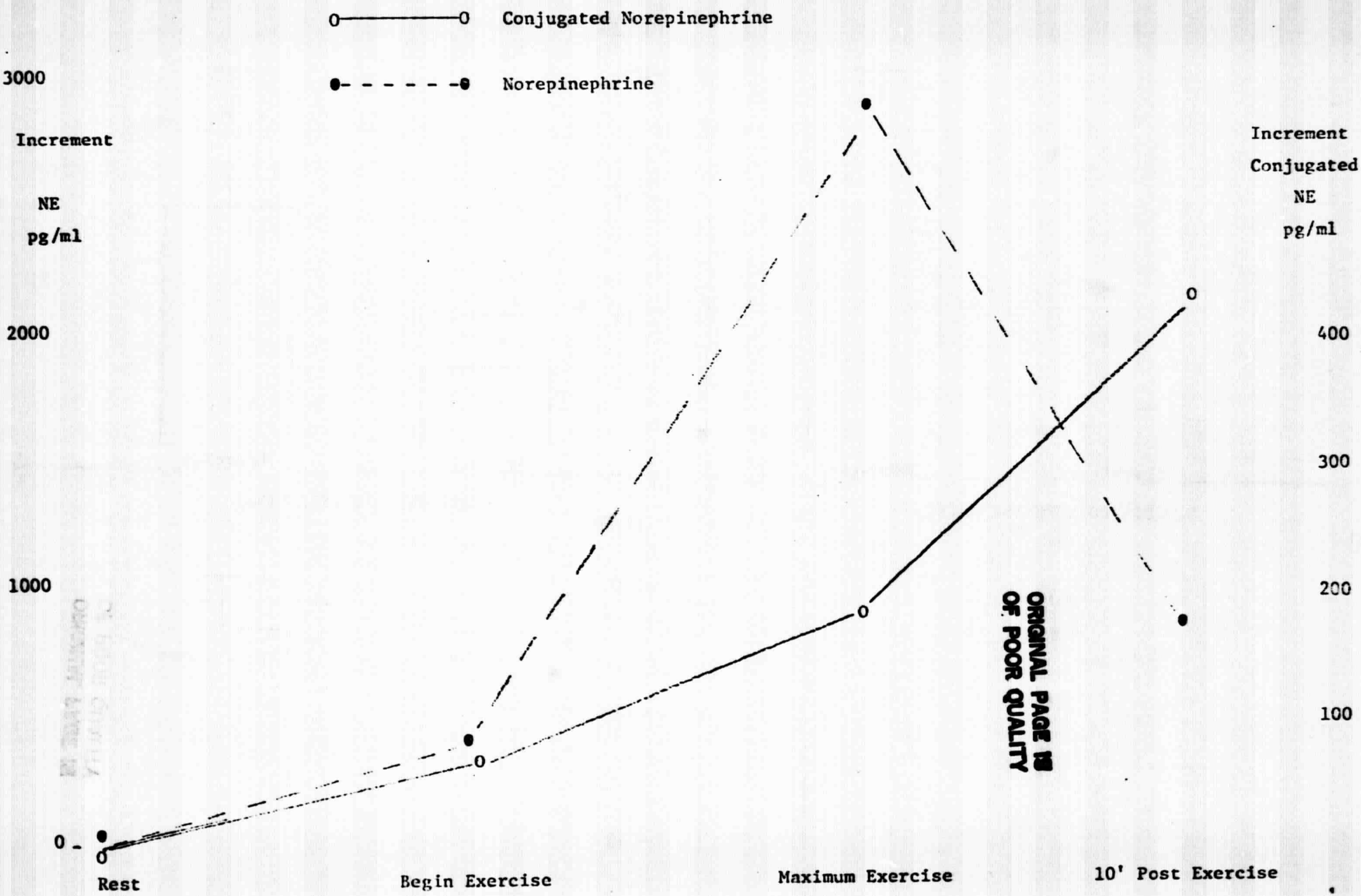
Conjugated Norepinephrine
Norepinephrine
3000
2000
1000
0
Increment
NE
pg/ml
Increment
Conjugated
NE
pg/ml
400
300
200
100
Rest
Begin Exercise
Maximum Exercise
10' Post Exercise

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FIGURE 4

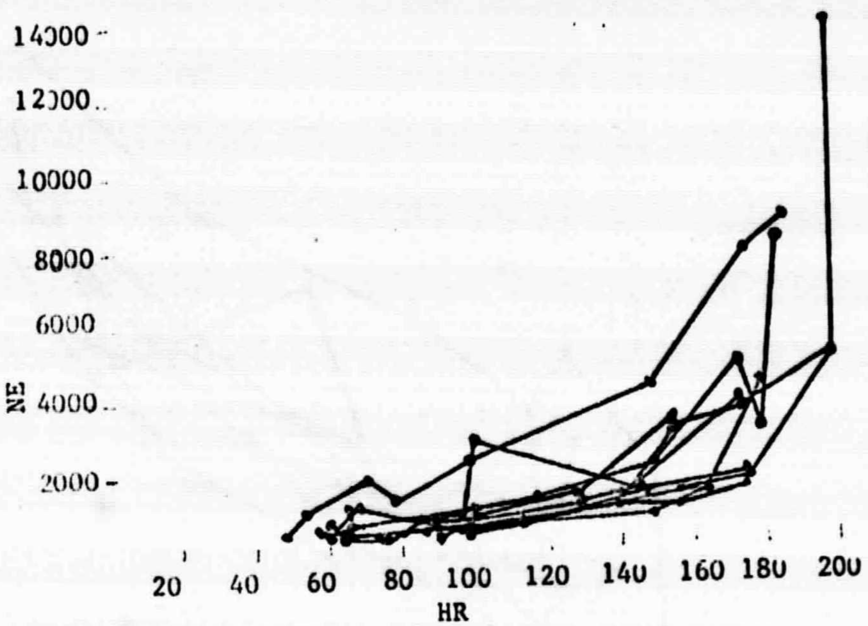

Figure 4: Plasma norepinephrine levels in pg/ml versus heart rate in 8 subjects exercised to their voluntary maximum on a treadmill. Blood levels were sampled every 3 minutes during and immediately following exercise.

FIGURE 5

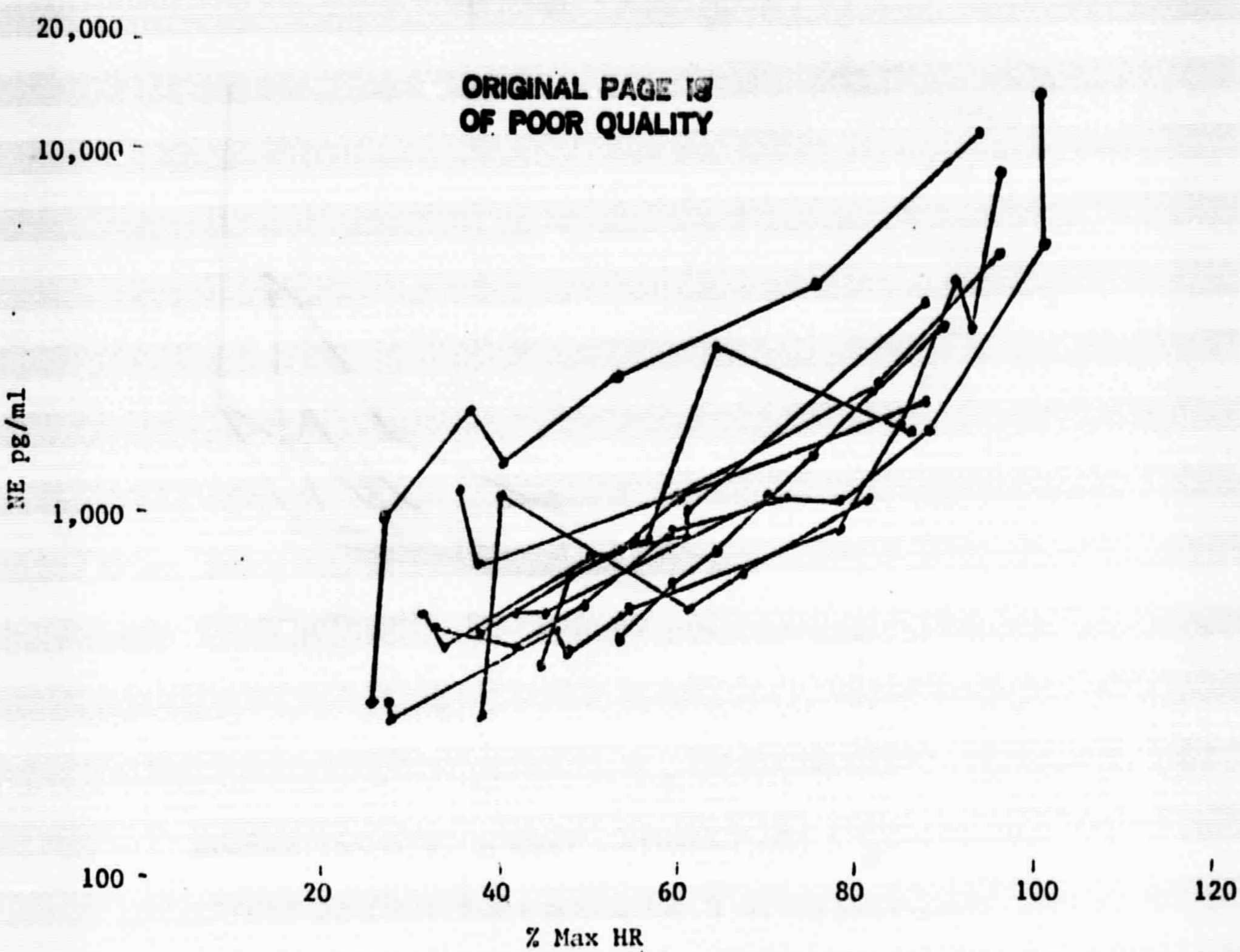

Figure 5: Norepinephrine levels in pg/ml versus percent maximum heart rate with norepinephrine shown on a logarithmic scale. Data is the same as that shown in Fig. 4.

scale versus the percent maximum heart rate. There is an exponential increase in NE as heart rate increases between 20% and 80% of the predicted maximum. However, above 80% of maximum heart rate, there is a supra-exponential increase. One individual maintained 100% of maximal heart rate for 3 minutes and doubled his plasma NE levels during those 3 minutes to 15,000 pg/ml. Representing NE levels in this manner is physiologically more valid than a simple linear graph, since NE interacts with alpha and beta receptors with a log dose-response curve that is linear over most of its effective range. Higher levels of NE, however, cause saturation of effector mechanisms so that NE elicits less of a response. This is seen in the range from 80% to 100% maximal heart rate. These considerations may make it worthwhile to routinely represent plasma catecholamines as a logarithm of their levels. Previous studies of catecholamine levels across population groups demonstrated that levels are not distributed in a normal bell-shaped curve, but are skewed toward one side. This skew can be corrected towards a normal distribution by taking the logarithm of catecholamine levels, which allows use of linear statistics to analyze differences in catecholamine levels.

Catecholamine Clearance Rates

This contract has allowed us to develop a technique for a sensitive assay for a variety of exogenous catecholamines in blood. We have taken advantage of this by infusing the synthetic catecholamine, isoproterenol, into man and then measuring blood levels of isoproterenol.

In Figure 6, blood levels of isoproterenol are represented on a logarithmic decay curve from 15 seconds to 8 minutes after a 30 minute infusion of isoproterenol was abruptly discontinued. The lower curve shows a decay rate of isoproterenol in subjects who were taking no medications, and the upper curve shows the decay rate of isoproterenol in the same subjects after they had taken propranolol 40 mg t.i.d. for one week. The half life of isoproterenol in the lower curve is 2.37 minutes and the half life of isoproterenol in the upper curve is 6.23 minutes. This dramatic increase in isoproterenol half life in response to the commonly used drug propranolol demonstrates the sensitivity of catecholamine clearance to commonly used drugs. This illustrates that plasma catecholamines represent not only the rate of entry of these amines from the sympathetic nervous system, but also the rate of clearance by $uptake_1$, $uptake_2$, metabolic, renal and hepatic mechanisms. It is reasonable to expect that decongestant drugs that interact with the sympathetic nervous system might alter catecholamine clearance rates and also that space flight, which alters blood volume and blood flow distribution, would alter catecholamine clearance. This is an important consideration when plasma catecholamines are used as an index of sympathetic nervous activity.

Over the past years, we have assisted Johnson Space Center in establishing the moderately difficult technology for assay of plasma catecholamines in small blood samples. The last two examples demonstrate that interpretation of catecholamine levels is as difficult as their measurement, and reveal that more basic research is necessary before a really intelligent interpretation of plasma catecholamines can be made.

FIGURE 6